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SOME STAGES IN THE DEVELOPMENT OF PELLIA EPIPHYLLA.

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Pellia epiphylla, one of the more common hepatics in this locality, grows on the ground in damp shaded places, its favorite location being on the banks of streams, either submerged or just above the water line, or more often extending several feet from the water's edge. It may be found in smaller patches on damp roadsides in close proximity to *Anthoceros* and *Blasia,* and scattered plants are sometimes found on boggy soil in pastures and swamps.

The slightly fleshy thallus has no suggestion of leaves, and varies from a simple oblong shape to one more or less sinuate, lobed and forked. (Pl. VIII, figs. 4, 5, 6.) The plants tend to grow in masses and sometimes will cover the soil for several feet if no other plant growth crowds them out. The ends of the plants overlap each other in shingle fashion (Pl. VII) doubtless to prevent too rapid evaporation of moisture.

This crowding together of the plants gives rise to a great variety of shapes. Those growing underneath or in more shaded positions take on a narrow, slender shape (Plate VIII, figs. 4a, 4b) while those having a greater exposed surface fork and fork again and broaden out horizontally (Figs. 5, 6a).

The color, texture and fruiting tendency of the thallus, varies somewhat according to the place of growth, this difference seeming to be governed by the amount of moisture available. The plants growing in drier locations are of a pale, dull-green color and the region of the costa is often characterized by a reddish or purplish tinge. These plants are thinner and more solid in texture than those growing in very moist situations. The plants growing close to the water's edge or on very wet soil have, in comparison, a rank luxuriant growth, the cell structure of the plant body being less compact. These plants have a richer, brighter green color with no trace of the purple tinge noted above.

Although the plants in moist situations have a more vigorous growth, they are far more likely to be sterile than those of drier locations, the latter being almost invariably well fruited. But when those in moist situations do form fruiting organs they are much farther advanced at the same season than those of a drier habitat.

The structure of the thallus is comparatively simple. A longitudinal section shows a slight degree of cell differentiation and a lack

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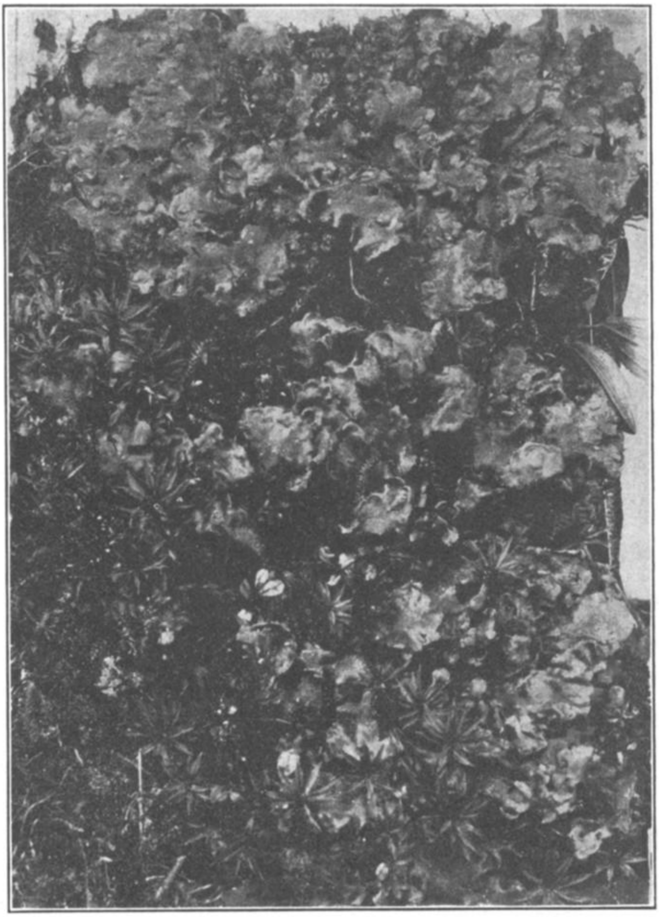

PLATE VII

Pellia epiphylla, nat. size. Collected and photographed Aug. 18.
Shows overlapping method of growth.
" average shape of thallus.
" position of involucre.
" " of antheridia.

of intercellular spaces. Fig. 8 illustrates a portion of a vertical section of a plant collected on July 10, which shows three somewhat clearly marked regions. There is an outer layer of epidermal cells, longer and narrower and more compact than the layers of chlorophyll-bearing cells below, which are bordered underneath by a double row of epidermal cells, from the lower of which the rhizoids develop. All the plants are provided with a thick mat of rhizoids which cling so tightly to the soil as to make it a very difficult matter to remove the particles of earth thoroughly enough for the safe cutting of material imbedded in paraffine.

Pellia epiphylla is monoecious, the archegonia being formed in groups just back of the growing point and the papilla-like antheridia being borne on the upper surface of the thallus, more abundantly close to the midrib and toward the growing point, where they seem to slant a little forward in that direction. Their number is very variable as can be seen from the photograph in Pl. VII. These antheridia appear very early in the life of the plant, oftentimes being found in abundance on the tiny new thalli that spring out at the edges of the old plants, shortly after the spores are shed in the middle of April, and these antheridial dots persist throughout the life of the plant, being plainly seen even after the thallus has become brown in color and has begun to die down and disintegrate.

The archegonia are formed in groups of varying numbers and are borne on the upper surface of the thallus just behind the growing point (Figs. 25, 26, 27b, 30*). They do not terminate its growth but usually after the appearance of the archegonia the forking of the costa begins at this point and two new divisions of the thallus develop more or less equally on either side, while the fertilized archegonium left at this point of division develops into a sporogonium (Figs. 4e, 4f). Archegonia may again appear just back of the tips of these new branches. As soon as the archegonia are formed a layer of tissue grows[1] out above and below (Figs. 26, 27a, 27b). The upper protective layer, called the involucre, grows forward horizontally until it

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1. The development of the archegonium is described in "The Structure and Development of Mosses and Ferns," by D. H. Campbell, Ph. D., as follows:

"After the archegonium mother cell is cut off it does not at once divide by vertical walls, but there is first cut off a pedicel, after which the upper cell undergoes the usual divisions." "The archegonium mother cell * * * is divided by a transverse wall into a basal cell and an outer one from which the archegonium itself develops. The divisions in this outer cell are remarkably uniform. Three vertical walls are first formed intersecting so as to enclose a central cell. In this central cell a transverse wall next cuts off a smaller, upper cell (cover cell), from a lower one. Subsequently the three (or in the Jungermanniaceae usually but two) first formed peripheral cells divide again vertically and by transverse walls in all of the peripheral cells, and somewhat later, in the central one. Also, the young archegonium is divided into two tiers, a lower one or venter and an upper one, the neck. The middle cell of the axial row, by a series of transverse walls gives rise to the row of neck canal cells and the lowermost cell divides into two, an upper one, the ventral canal cell, and a lower one, the egg." p. 16 & p. 90. 1895.

*In Sept. no.

reaches the end of the group of archegonia (Pl. VIII, figs. 4d, 6b, 6c). This fold of tissue remains lifted up from the thallus for a time, perhaps until after fertilization has taken place. *Later* these involucres are pressed tightly down upon the thallus underneath, securely enclosing the sporogonium. The number of archegonia formed seems to vary considerably, sometimes there are only nine or ten, and again there may be more than twenty (Fig. 29a). But ordinarily only one develops into a sporogonium, although twice I have found two full-grown sporogonia of equal size growing together under the same involucre.

After fertilization the archegonia develop through the summer and autumn months into sporogonia which reach their full development by the last of October. These are composed of a foot, stalk (seta) and capsule, and remain hidden away under the tightly fitting involucres during the winter months until April, when the spores are ripe. Then the stalk which has remained short, suddenly elongates vertically to the height of an inch or more (Figs. 7a, 7b) the walls [1] of the capsule split vertically into four valves and the spores are shed. The foot is the basal portion of the stalk and in *Pellia* "is very distinct and forms a pointed conical cap whose edges overlap the base of the seta [2]," (Figs. 37, 38, 39, 43, 44).

The study in preparation for this paper was undertaken with the purpose of finding out when the more important stages in the life history of *Pellia epiphylla* occurred.

For this reason material has been collected in every month of the year, beginning in April when the spores are shed and the new plants begin their growth and continuing until the following April, when the life cycle has been completed. Collecting for this work was begun in 1908 and was continued during 1909 and 1910 as opportunity offered. Collections have been made from different localities about the city of Worcester, Mass., where *Pellia epiphylla* was to be found in fairly large amounts. For fixing agents several different chromacetic solutions were used with satisfactory results. The formula best suited to the earlier stages (those collected from April-July) was composed of 1 gram. chromic acid, 4 cc. glacial acetic acid, 100 cc. H_2O.

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1. "The growth of the seta after the spores are ripe is extremely rapid, but consists entirely in a simple elongation of the cells. Askenasi (Wachstum der Fruchstiele von *Pellia epiphylla*. Bot. Zeit. 1874, p. 237) has investigated this in *Pellia epiphylla* and states that in three or four days the seta increases in length from about 1 mm. to in some cases as much as 80 mm., and that this extraordinary extension is at the expense of the starch which the outer cells of the young seta contain in great abundance, but which disappears completely during the elongation of the seta."
D. H. Campbell, l. c. p. 93.

2. D. H. Campbell, l. c. p. 92.

While the following formula, 70 cc. one per cent. chromic acid, ½ cc. glacial acetic acid, 30 cc. H_2O, gave better results with the different stages of the sporogonia. Carnoy's fixing fluid was used twice in order to save time, and although the material fixed in this solution took a very brilliant stain with the saffranin gentianviolet combination, more or less shrinkage resulted. This, however, might have been prevented if the material had not been hurried too rapidly through the infiltrating and imbedding process.

The plants were either put into the fixing solution in the field or soon after carrying them home. When such a delay was necessary the material was kept in a tightly covered tin box until placed in the chromacetic solution. In the earlier stages after removing the dirt from the rhizoids with needles, entire plants may be placed in the fixing solution. But after July, when the young sporogonia begin to develop, the thallus should be trimmed down nearly to the sporogonium. The capsules should be pricked and the surrounding membrane removed, otherwise bubbles will form making it impossible for the fixing solution to penetrate, and later the paraffine, thus making the infiltration process a failure.

After fixing for about 24 hours, the material was washed in running water for about 12 hours and then was carried through the usual solutions of alcohol, viz.: 15 per cent., 35 per cent., 50 per cent., 70 per cent., comparatively short periods (3 hours) being sufficient for the weaker alcohols. In 70 per cent. alcohol much of the material had to remain for a long time, from several months even to a year.

When ready for imbedding the material was carried from 70 per cent. through 85 per cent., 95 per cent , two changes of absolute alcohol and through the three solutions of absolute alcohol and xylol into pure xylol and was finally imbedded in paraffine melting at 54° C. The sections were cut with a Minot rotary microtome, being three, four or five microns in thickness.

In the earlier stages great trouble was experienced from shrinkage of the tissues. After the loss of much time and good material it became evident that the difficulty, though caused partly by insufficient washing, was chiefly due to allowing for too short periods in the absolute alcohol and xylol solutions and also to hastening the process of infiltration. Moreover, when all had gone well up to the last solution in xylol everything was sometimes ruined by adding the paraffine in too large pieces. Even in the most refractory material, shrinkage was always avoided by letting it remain a long time (24 hrs. or longer) in the absolute alcohol-xylol solutions and then putting only the smallest, thinnest shavings of paraffine into the pure xylol, letting each dissolve before adding the next.

After many trials and experiments I worked out the following schedule which gave uniformly good results.

Schedule for imbedding *Pellia epiphylla.*

Fixing solution 24 hours or more.		
Wash in running water 12 hours or more.		
15 per cent. alcohol,		3-6 hours.
35 per cent.	"	3-6 hours.
50 per cent.	"	3-6 hours.
70 per cent.	"	6 hours-anytime.
85 per cent.	"	4-12 hours.
95 per cent.	"	12-24 hours.
abs. alcohol	1st sol.	12 hours or more.
" "	2nd "	12 hrs. or more.
abs. "	($\frac{2}{3}$) + xylol ($\frac{1}{3}$)	24 hrs. or longer.
" "	($\frac{1}{2}$) + " ($\frac{1}{2}$)	24 hrs. or longer.
" "	($\frac{1}{3}$) + " ($\frac{2}{3}$)	24 hours or longer.

Too much emphasis cannot be laid on the necessity of long periods in the last three solutions.

xylol 1st sol. 12 hrs. or longer. Drain thoroughly.
xylol 2nd sol. 2 or 3 hours or longer.

Then begin to add paraffine *very slowly in minute* shavings. As the solution becomes saturated larger pieces may be added with greater frequency. The material seemed to be benefited by remaining some time in the xylol-paraffine mixture. A week or ten days gave good results, and apparently a longer time would do no harm.

Soft paraffine melting at 37° C. was used and the greatest care was necessary to see that the temperature did not rise above that point. The shortest time that it was safe to leave the material in the paraffine oven was 24 hours, and during that time, the paraffine solution should be poured off at least three times and renewed with shavings of paraffine of the same melting point. A longer time than twenty-four hours is desirable and the more times the paraffine is changed the better the result will be.

In transferring from soft to hard paraffine, again the greatest care must be taken that the temperature of the latter shall not be too high, or shrinkage will result and all the painstaking work of previous days or weeks will be lost. One successful method is to heat the hard paraffine on an iron stand and allow it barely to reach the melting point. and then pour out into a paper tray. The material in the soft paraffine can then be taken from the oven and transferred to this and with hot needles each piece can be carefully oriented. The paper trays should then immediately be floated on cold water until thoroughly cooled. If they are taken out of the water and allowed to dry thoroughly, the paper will easily peel off from the paraffine cakes

without having recourse to the inside coating of glycerine which makes them so unpleasant to handle. The specimens are then ready for sectioning in an hour's time.

Hard paraffine if melted over several times before being used for imbedding will cut very much better than that taken directly from the new cake. But the greatest care must be taken not to let it get too hot by letting the temperature rise too much above the melting point.

The attempt to use xynthol instead of absolute alcohol resulted in failure, for material in good condition up to the time of transference to a xynthol solution became so shrunken and dried that it was worthless. Neither did xynthol work well in dehydrating the slides in the process of staining.

In regard to the stains that were used, Delafield's haematoxylin was found to be most satisfactory for antheridia and archegonia and Fleming's triple stain of saffranin, gentianviolet and orange G for the later stages. For this work over forty different collections of *Pellia epiphylla* have been made, the number for each month depending upon the activities of the plant, seven collections being made in June, against one each in the months of December, February and March.

These different collections will now be considered in order, beginning with the earliest date on which the new season's growth was found.

April 15 one of the best collecting grounds of the city was visited. Here within the space of a few feet quite a variety of stages was found. The plants on the bank a few feet back from the river's edge were dried down and the sporogonia hardly protruded from under their protecting membranes. On a tussock directly over the water was a mass of plants with sporogonia that had pushed up on stems an inch or more in length with capsules that seemed just ready to open. Others had already opened and had shed their spores, only the brown tufts of fixed elaters at the center of the base of the capsule being left. Between these extremes of dry and moist conditions the sporogonia were found in various stages. Some were just ready to shed their spores, others had pushed up only a short distance, others were just protruding beyond the involucre. Those whose stems seemed to have grown to full height, or whose spores had been discharged showed a thallus much reduced. Generally nothing was left of the old plant but a thin narrow band, dark brown in color, and the sporogonium instead of being some distance back from the growing point now seemed to be at the very tip of the thallus (Figs. 7a, 7b). New bright-green shoots, more or less folded and curved, were to be seen springing out from the edges of the old plants which seemed about to die down and disintegrate (Figs. 2, a, b, c, d, e).

The next collection was made on April 21, at the same place. Almost exactly the same variety of stages of sporogonial development

was to be seen. Near to the water's edge were the long stems of the capsules surmounted by their brown tufts of elaters. Then farther and farther back from the water were all lengths of stems of capsules back to the little sporogonia that had not as yet pushed out from under their protecting involucres and seemed to have no intention of leaving winter quarters. But all the thalli showed new growth. New shoots had developed at the edges of the old plants, and on many of these the antheridial dots were already evident to the naked eye. These new plants were so tender and delicate that it was very difficult to remove the dirt particles without utterly destroying the specimens. They gave great trouble in the imbedding process, either by shrinking, or by curling up and folding together, so that when they were finally in place in the hard paraffine it was nearly impossible to cut a good longitudinal section. However, patience and perseverance made it possible to get some satisfactory sections which showed various stages in the development of the antheridia. Fig 29 represents a nearly median longitudinal section through the growing point and shows four stages of antheridial development, the most immature being near the growing point. The farther away from the growing point they are found the more maturity of development they will have.

In regard to the development of the antheridia, Campbell says [1] that each arises from a single superficial cell (Figs. 10 & 10a) which first divides into a stalk cell and the antheridium mother cell (fig. 11). The stalk later becomes multicellular, while the antheridium mother cell is divided into two equal parts by the formation of a vertical wall (fig. 12). [2] "Next in each of these, two walls arise intersecting each other as well as the median wall and divide each half of the antheridium into three cells, two peripheral ones and a central one. The peripheral ones do not reach to the top of the antheridium and next a periclinal wall is formed near the top of the central cells by which a third peripheral cell is formed in each half of the antheridium, which now consists of two central cells and six peripheral ones." "The latter [3] divide only a few times**." "The inner cells give rise to a very large number of sperm cells."

Only a few of the stages of development of the antheridia were found in this material, but these few were found repeatedly in all the material gathered in April and May. Figs. 10 and 10a show the cell from which the antheridium is to develop, plainly distinguished by its large nucleus and the deepness of the stain. Fig. 11 shows the two-celled stage with the stalk cell and the antheridium mother cell having a large nucleus and taking a deep stain. Fig. 12 shows the first division of this antheridium mother cell into two cells by means of a vertical wall. No other stages were found until the more advanced one in which the central group of cells, which by division will form

1. D. H. Campbell, l. c. pp. 16, 92 and 85. 2. l. c. p. 85. 3. l. c. p. 16.

the sperm cells, has been differentiated from the outer single layer of sterile cells which form the wall of the antheridium (Figs. 13 and 14). Fig. 15 shows what appears to be a mature antheridium, the central space being occupied by many sperm cells. Thus it would seem that the antheridia may be fully formed by the latter part of April.

By means of the division of cells adjacent to the young antheridium it soon becomes surrounded by a partition and each becomes sunk in a little cavity or pocket (Figs. 11, 13, 14, 15).

On April 28, more material was collected from the same place. The young shoots showed considerable growth in a week's time. Again there was a great difference to be seen between the plants growing close to the water and those on the drier bank a few feet away. The former were much larger in size and more rank in growth, while the latter were small in size and thin in substance. The papilla-like antheridia showed plainly. All the capsules had opened, but the stems surmounted by the tuft of elaters were still standing. Sections through this material showed no special difference from that gathered on April 21, so no figure is given.

May 6 was the next collecting day, and this time another locality was visited, where the plants were growing at the river's edge. These plants showed a marked increase in size over the preceding, and were very rank in growth and bright green in color. Sections of this material showed no marked advance over that gathered earlier, only there was a greater proportion of the more mature antheridia and fewer of the early stages were to be found.

[TO BE CONTINUED.]

EXPLANATION OF FIGURES

These drawings were made with a Bausch and Lomb microscope, using ⅔ and ⅙ objectives, and 1 inch and ¾ inch eyepieces.

The sections were four or five microns in thickness.

Figs. 8—31 were stained with Delafield's haematoxylin.

Figs. 31—51 were stained with the saffranin gentianviolet and Orange G combinations.

EXPLANATION OF PLATE VIII

Fig. 2. a, b, c, d, e. New thalli springing out from edge of old plants, collected April 21. c_1, d_1, e_1. Old plants.

Fig. 2. f. Characteristic shape of thallus, June 28.

Fig. 3. a, b, c. Varying shapes of thallus, July 4. a_1, c_1. Old plants.

Fig. 4. a, b, c, d, e f. Different shapes of plants, Aug. 16. c_1. Old plant. e & f. show position and appearance of young sporogonium at this time with involucre dissected away.

Fig. 5. Thallus collected Aug. 18, shows forking tendency when no surrounding plants interfere with growth.

Fig. 6. a, b, c. Collected Aug. 6.

6, a. Characteristic shape growing on wet leaves near the water.

6, b & c. An average shape of thallus, shows position of antheridia and involucre.

Fig. 7. a & b. Plants collected April 15.

c. Calyptra.

Capsule has been pushed through this by the lengthening of seta and is just ready to open and shed the spores. Thallus much dried down and reduced in size.

Figs. 1—7. All nat. size.

Fig. 8. Vertical section through thallus × 80, collected July 10.

e, epidermal cells. p, parenchyma cells. r, rhizoids. 5μ thick.

Fig. 9. Young sporogonium, Aug. 17, × 20, capsule plainly differentiated, seta hardly at all. n, withered neck of archegonium.

EXPLANATION OF PLATE IX

DEVELOPMENT OF ANTHERIDIA

All median longitudinal sections and × 233.

Fig. 10. Earliest stage, single superficial cell, May 29 (4μ).

Fig. 10, a. Same stage as 10. Apr. 21 (4μ).

Fig. 11. Two-celled stage, stalk cell and antheridium mother cell, Apr. 21 (4μ).

Fig. 12. First division of the antheridium mother cell by vertical wall, Apr. 21 (4μ).

Fig. 13. More mature stage, May 29. Shows characteristic shape of stalked antheridium enclosed in protecting cavity. Central cells by division will form sperm cells surrounded by layer of sterile cells (5μ).

Fig. 14. Somewhat more advanced stage than fig. 13, Apr. 21 (4μ).

Fig. 15. Mature antheridium, Apr. 21 (4μ).

DEVELOPMENT OF ARCHEGONIA

All × 233.

Fig. 16. Two early stages of archegonia from Fig. 26, June 14, median long. section.

16a. Single superficial cell ; one celled stage.

16b. Two-celled stage, archegonium mother cell ready to divide into two.

Fig. 17. Two-celled stage, July 10, median long. section.

Fig. 18. First division of archegonium mother cell, June 14, median long. section.

Figs. 19 & 20. June 14.

Shows central cell set off from peripheral cells, optical section.

Figs. 21. 22, 23. Median longitudinal sections of more advanced stages, July 10.

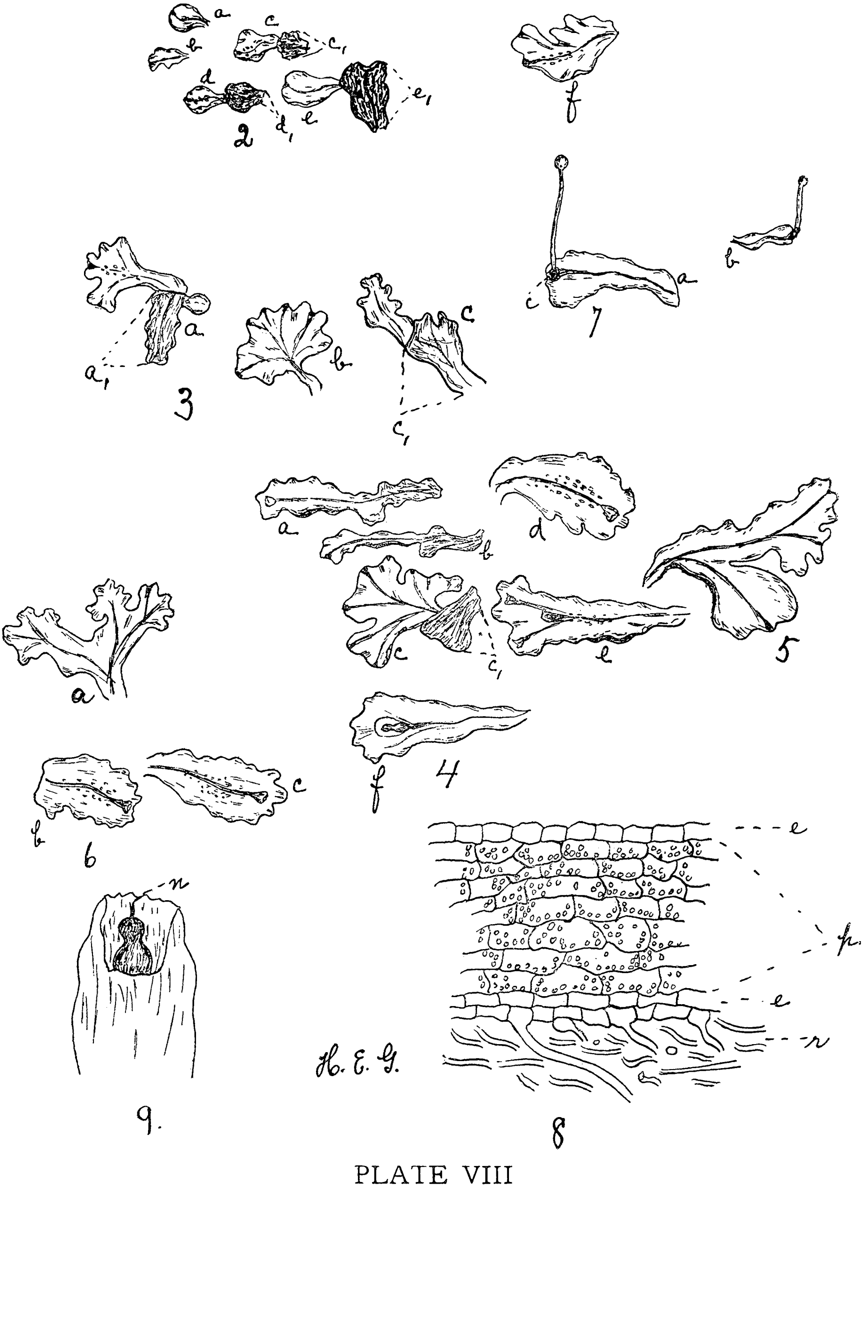

PLATE VIII

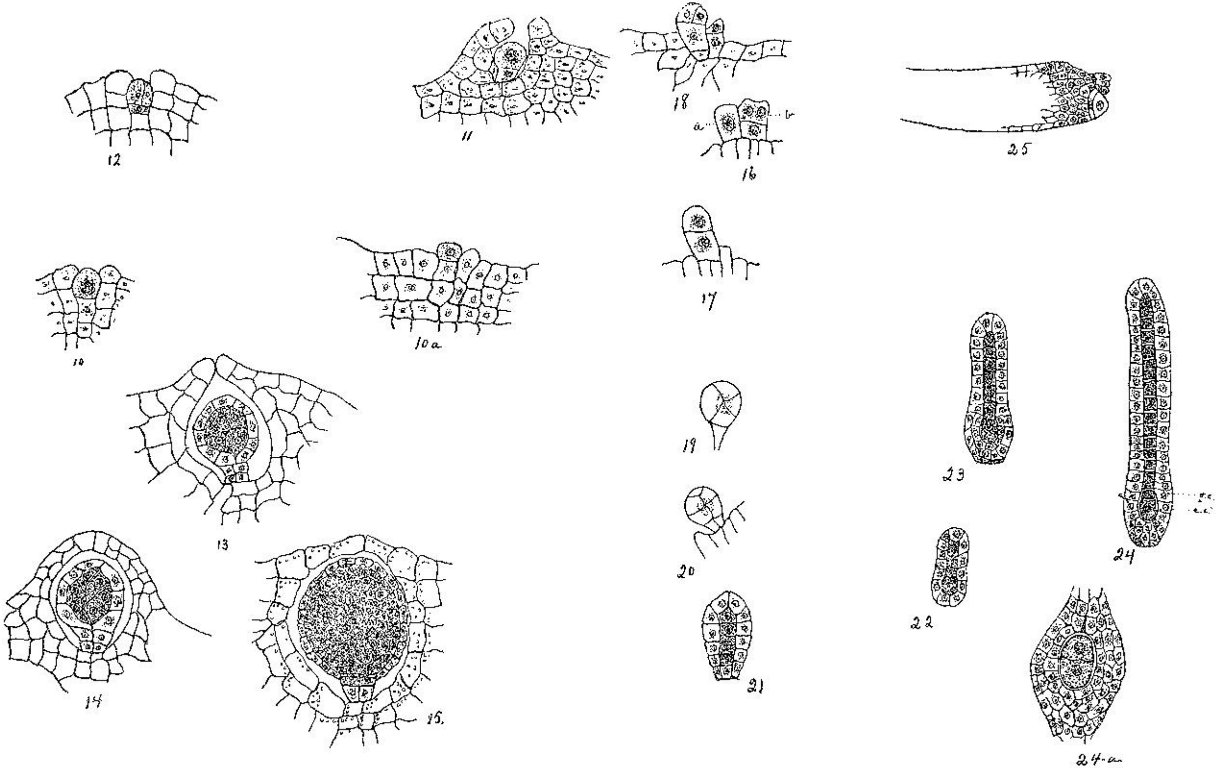

PLATE IX

Fig. 24. Mature archegonium, median long. section, June 14. v. c., ventral canal cell. e. c., egg cell; and 16 canal cells.

Fig. 25. median long. sect. through growing point of thallus $\times$ 110. June 14. Young archegonia. In drier soil.

Fig. 24a. Median longitudinal section through venter of archegonium, July 26. Young embryo, 2-celled stage. Increase of cell division in walls of venter.

AUSTINELLA gen. nov.

R. S. WILLIAMS

Dioicous: stems with few radicles, a distinct central strand and brown outer walls composed of about three rows of thick-walled cells; leaves spreading-flexuous, from a very broad, imbricate base abruptly narrowed to a narrowly lanceolate, grooved point; leaf margins flat or mostly so and serrulate above; cells of leaf lamina distinct and nearly or quite smooth on both sides; costa stout, semiterete, excurrent, somewhat rough on the back above, in cross-section near middle showing 9 or 10 guide-cells with stereid bands and more or less numerous accessory guide-cells both above and below, outer cells somewhat differentiated; cells in lower leaf linear to somewhat elongate hexagonal with more or less colored, mostly thickened, rarely slightly pitted walls, the alar not distinctly differentiated; the upper leaf of angular cells, nearly square to 2 or 3 times longer than wide, the narrow upper blade or margin of a double thickness of cells.

This genus is near *Trichostomum* but has a broad, clasping base to the leaf, and cells of upper leaf angular and not papillose.

Austinella Rauei (Aust.) gen. nov.

Syrrhopodon ? Rauei Aust. Bull. Torr. Bot. Club, **6:** 74. 1876.
Dicranum fulvum Hook. fide Lesq. & James, Manual, 70. 1884.
Dicranodontium inundatum Small, Exsiccati, Mosses of Southern U. S. (nomen undum).

Dioicous, male flowers, 2 or 3 in number, scattered along upper stem on very short, axillary branches, the outer perigonial leaves abruptly narrowed and spreading from a short, broad base, the inner much shorter, entire, enclosing about 6 antheridia with few, shorter paraphyses: in extensive mats, dull yellowish green at the surface, dark brown within; stems with central strand and about 3 rows of thick walled outer cells, slightly radiculose below, somewhat branching above, up to 2 or 3 cm. high; upper stem leaves 4 or 5 mm. long, spreading-flexuous, scarcely crispate, from a clasping, ovate or obovate base scarcely 1 mm. long with margin not quite entire, abruptly narrowed to a somewhat grooved, lanceolate point 3 or 4 times longer, irregularly serrulate on the flat margins about one-half down, smooth